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LINE-1 Elements at the Sites of Molecular Rearrangements in Alport Syndrome–Diffuse Leiomyomatosis

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Summary

Deletions encompassing the 5′ termini of the paired type IV collagen genes *COL4A5* and *COL4A6* on chromosome Xq22 give rise to Alport syndrome (AS) and associated diffuse leiomyomatosis (DL), a syndrome of disseminated smooth-muscle tumors involving the esophagus, large airways, and female reproductive tract. In this study, we report isolation and characterization of two deletion junctions. The first, in a patient described elsewhere, arose by a nonhomologous recombination event fusing a LINE-1 (L1) repetitive element in intron 1 of *COL4A5* to intron 2 of *COL4A6*, resulting in a 13.4-kb deletion. The second, in a previously undescribed family, arose by unequal homologous recombination between the same L1 and a colinear L1 element in intron 2 of *COL4A6*, resulting in a >40-kb deletion. L1 elements have contributed to the emergence of this locus as a site of frequent recombinations by diverse mechanisms. These give rise to AS-DL by disruption of type IV collagen and perhaps other as yet unidentified genes, evidenced by deletions as small as 13.4 kb.

Introduction

Alport syndrome (AS) or "hereditary nephritis" (MIM 104200, 301050) is a predominantly X-linked disorder that is marked by hematuria and progressive renal failure in affected males and is often accompanied by hearing loss and ocular abnormalities (Alport 1927; Gregory and Atkin 1993). Female carriers generally exhibit a milder phenotype, although exceptions are well known (Hasstedt et al. 1986). A small subset of affected individuals manifest an associated syndrome called "diffuse leiomyomatosis" (DL), characterized by disseminated smooth-muscle tumors of the esophagus, upper airways, and female genitalia (Cochat et al. 1988; Garcia-Torres and Orozco 1993). Leiomyomas are equally penetrant in males and females and can present sporadically (Heidet et al. 1998).

Most cases of X-linked AS are attributable to mutations in the *COL4A5* gene, which is arranged head-to-head on chromosome Xq22 with the closely related *COL4A6* gene. Together with the gene pairs *COL4A1-COL4A2* and *COL4A3-COL4A4*, which are arranged head-to-head on chromosomes 13 and 2, respectively, *COL4A5* and *COL4A6* encode the α1-α6 chains of type IV collagen, which form triple-helical monomers of variable and tissue-specific composition and function as a lattice network in basement membranes (reviewed in Hudson et al. 1993; Timpl and Brown 1996). It is likely that the genomic arrangement of the type IV collagen genes arose by a series of duplications from an ancestral gene (Zhou et al. 1994). To date, >60 pathogenic mutations in *COL4A5* have been described, including deletions, insertions, rearrangements, and single base-pair substitutions (Tryggvason 1996). Patients with AS-DL characteristically have large deletions in the *COL4A5-COL4A6* locus, which encompasses the 5′-most exons of *COL4A5* and *COL4A6* and their intergenic region (Zhou et al. 1993). In cases examined to date, there is loss of immunoreactivity to the α5 and α6 chains in basement membranes (Heidet et al. 1997*a*).

The basis for smooth-muscle tumors in AS-DL is unknown. We and others have postulated that DL arises not strictly as a result of loss of the α5 and α6 chains but as a result of disruption of an as yet unidentified gene(s). Two major lines of evidence point to this likelihood. First, cases of AS arising from mutations in *COL4A5* generally are associated with loss of immunoreactivity for the α3-α6 chains in kidney and other organs (Kleppel et al. 1989; Nakanishi et al. 1994; Peissel et al. 1995), implying that loss of the α5 and α6 chains—although perhaps necessary for DL—is not sufficient. Second, deletions in the *COL4A5-COL4A6* lo-

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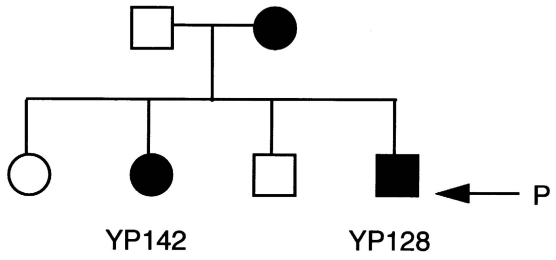

Figure 1 Pedigree of the YP family. The proband (P) YP128 is indicated by an arrow (←). The blackened symbols refer to affected family members, as outlined in Patients and Methods.

cus extending beyond the third exon of *COL4A6* are associated with AS but not with DL (Heidet et al. 1995, 1997*b*). This finding is consistent with a scheme in which disruption of gene(s) or genetic regulatory element(s) in the locus gives rise to smooth-muscle tumors by gain of function, in a manner abrogated by more extensive deletions.

Long-range mapping studies indicate breakpoint variability in AS-DL–associated deletions (Heidet et al. 1995, 1997*b*). Correlations between the extent of deletions with expression of the DL phenotype have been used to map a "critical" region for additional genes to a 90-kb region encompassing exon 3 of *COL4A6* (Heidet et al. 1997*b*). In an alternative approach, we postulated that the smallest known deletions giving rise to DL might directly disrupt or cause dysregulation of candidate gene(s) for DL. As such, we report refined mapping and molecular cloning of the deletion junction in a patient with a deletion described elsewhere (Renieri et al. 1994). In addition, we report a deletion junction in a previously undescribed family with AS-DL, and we use this to characterize further the basis for diverse recombination events generating deletions in this locus. Our findings provide greater molecular detail of the locus and highlight factors that may predispose uniquely to recombination events giving rise to AS-DL.

Patients and Methods

Patients

Patient DON has been described elsewhere (Renieri et al. 1994). In brief, he is an adopted 30-year-old man, diagnosed with AS at 6 years of age, who progressed to end-stage renal disease by age 16 years and subsequently underwent renal transplantation. Esophageal leiomyomas were diagnosed during his early twenties. The patient has bilateral high-tone hearing loss and bilateral cataracts with right lenticonus.

Patient YP128 is a 29-year-old man, from the pedigree shown in figure 1. Hematuria was first detected at age 11 years. Renal biopsy specimens obtained at age 16 years showed splitting and fragmentation of the glomerular basement membrane at the electron microscopic level, which is characteristic of AS. Progression to end-stage renal disease occurred by age 25 years. Esophageal leiomyomas were resected partially at age 17 years. Bilateral cataracts and sensorineural hearing loss were documented during the patient's twenties. The patient's sister, YP142, progressed to end-stage renal disease by age 30 years. She underwent partial resection of esophageal leiomyomas and resection and reconstructive surgery for vulvar leiomyomas during her teens. During her twenties, she underwent anal sphincterotomy and excision of the internal anal sphincter, for severe constipation secondary to hypertrophy of these structures. She has bilateral cataracts and asthma. The mother of these patients is age 60 years. She underwent esophagomyotomy at age 40 years. She has microscopic hematuria and a serum creatinine level of 1.3 mg/dl. She has no clinically evident hearing loss or ocular abnormalities.

Southern Analysis

Epstein-Barr virus–transformed cell lines were prepared from patient blood samples and genomic DNA isolated by use of standard techniques (Dracopoli et al. 1997). After overnight digestion, DNA samples (5 μg) were separated on 0.8% agarose gels and transferred to Magnagraph nylon membranes (Micron Separations). Probes were prepared by ^{32}P-dCTP random labeling, with the following templates. D2 from intron 1 of *COL4A5* is a 710-bp PCR product obtained by use of primers 5′ CAAGAATGTTTCTGACTTGGC 3′ and 5′ TCTACATTACAACTAACCTTATC 3′. D1, Y1, and Y2

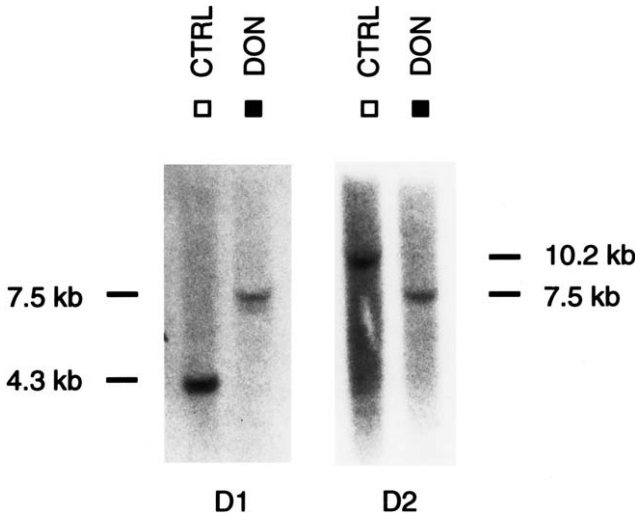

Figure 2 Southern analysis of the DON deletion. Hybridization with use of probes D1 and D2 demonstrated an abnormal 7.5-kb *Sac*I fragment in genomic DNA from the DON patient, in contrast to the expected 4.3- and 10.2-kb fragments (see fig. 3 for map).

from intron 2 of *COL4A6* are 330-bp *Sau*3AI, 1.1-kb *Hin*dIII, and 260-bp *Stu*I-*Sac*I fragments, respectively. The α6 cDNA fragment JZ-3-FR5, covering one of two alternative first exons to exon 4 of *COL4A6,* has been described elsewhere (Zhou et al. 1993). Hybridization in 50% formamide-containing buffer and subsequent washings were carried out by use of standard techniques (Ausubel et al. 1987). Bands were visualized by means of autoradiography.

PCR Amplification

Cosmid clone U176D11 (Renieri et al. 1994), encompassing the 5′ ends of the *COL4A5* and *COL4A6* genes, was partially sequenced for the purpose of designing primers for PCR amplification across patient deletion junctions. For patient DON, a first reaction was performed on genomic DNA by use of primers DF1 and DR1 (table 1 and fig. 3), followed by a nested reaction using primers DF2 and DR2. Patient YP128 has a *COL4A5* breakpoint in intron 1 (see Results). The *COL4A6* breakpoint region was isolated by inverse PCR (Triglia et al. 1988). Total genomic DNA was digested with *Eco*RI, circularized with T4 ligase, and amplified with outward-facing primers YF1 and YR1 (table 1 and fig. 4), yielding a 2.8-kb product, which was cloned and sequenced. Flanking sequence was also isolated by use of inverse PCR. To extend in the *COL4A6* direction, genomic DNA from patient YP128 was digested with *Sac*I, circularized, and amplified with outward-facing primers YF1 and YR2, the latter from the *COL4A6* breakpoint region. To extend in the *COL4A5* direction, genomic DNA from an unaffected individual was digested with *Sac*I, circularized, and amplified by use of YF2 and YR3, yielding a 4.5-kb product, which was cloned as an *Eco*RI-*Sac*I restriction fragment. Amplification across the YP128 deletion junction was performed with primers YF2 and YR1.

Immunofluorescence

Immunofluorescence was performed as described elsewhere (Peissel et al. 1995). In brief, skin biopsy materials obtained from YP128 and a control individual were maintained in OCT at −80°C. Sections of 2–4-μm thickness were cut at −20°C and denatured by use of urea-glycine (pH 3) for 1 h at 4°C. Staining for the C-terminal NC1 domains of the α1, α5, and α6 chains of type IV collagen was performed with primary antibodies, as described elsewhere (Peissel et al. 1995), and with appropriate fluorophore-conjugated secondary antibodies. Staining was visualized, after mounting, by means of epifluorescent illumination.

Results

Characterization of Deletion Junctions

Southern analysis of genomic DNA from patient DON has been used broadly to define a 15–19-kb deletion that encompasses the first exon of *COL4A5* (E1), two alternative first exons of *COL4A6* (E1a and E1b), and the second exon of *COL4A6* (E2) (Renieri et al. 1994). We used genomic probes D1 and D2, from the deletion-flanking regions, to identify an abnormal 7.5-kb *Sac*I fragment and, thus, narrowed the deletion to 13.4 kb (fig. 2). This is one of the smallest known deletions associated with AS-DL (see Heidet et al. 1995). PCR amplification across the deletion junction revealed fusion of a 5′-truncated L1 repetitive element in intron 1 of *COL4A5* to a nonrepetitive sequence in intron 2 of *COL4A6,* with interposition of a 70-bp poly-A/T stretch (fig. 3). The L1 element has been described elsewhere (Ueki et al. 1998). It contains nucleotides 3617–5979 of an arbitrary full-length L1 consensus sequence (Genbank accession number U93574) and therein lacks the two open reading frames associated with functional elements (Sassaman et al. 1997). The *COL4A5* breakpoint is in its poly-A tail, whereas the *COL4A6* breakpoint is in a poly-A/T stretch 16 bp in length, which is arranged tail-to-tail on the same strand.

In preliminary Southern analysis of genomic DNA from patients Y128 and Y142, using probe JZ-3-FR5, we confirmed a characteristic deletion involving exons E1 of *COL4A5* and E1a, E1b, and E2 of *COL4A6,* with

Table 1

PCR Primer Sequences

Template	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
DON:		
Undigested	DF1 TCAATCAGACCTTGATTTGAGTGCAGC	DR1 ATCGCCAAGTCAATCCTAAGCCA
	DF2 AGGGGGCAAAGTGTGAAG	DR2 ACAAATCTACAACTATCTGATC
YP128:		
*Eco*RI-digested, circularized	YF1 CAACATACCCATGAGGTCCCTTTGG	YR1 TGTAGCAAGAATGTTTCTGACTTGGC
*Sac*I-digested, circularized	YF1 TGTAGCAAGAATGTTTCTGACTTGGC	YR2 CGTAGTCAAGTCTATACTGACTAAGG
Undigested	YF2 GGCAAGCACAGAAGCAGAAAGGC	YR1 TGTAGCAAGAATGTTTCTGACTTGGC
Wild-type:		
*Sac*I-digested, linearized	YF2 GGCAAGCACAGAAGCAGAAAGGC	YR3 ACATCAACAAATCCAAATGGACATCC

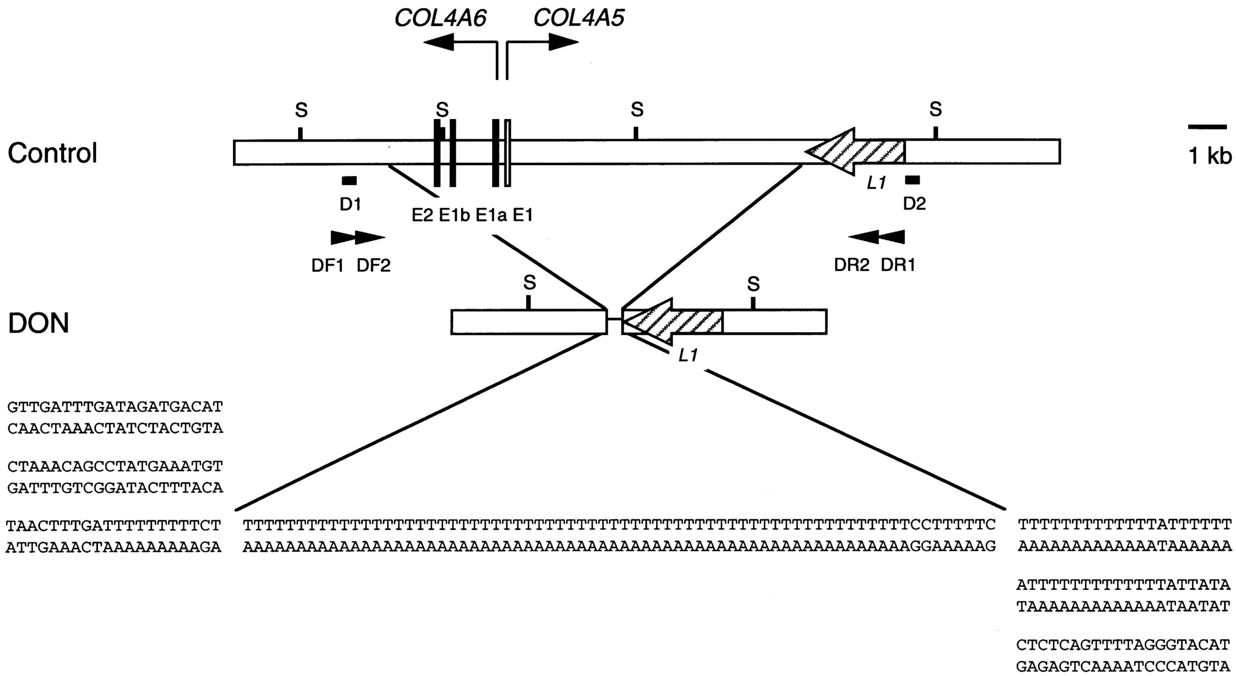

Figure 3 Deletion junction in the DON patient. The 5′-most exons of *COL4A5* (*unblackened bar*) and *COL4A6* (*blackened bars*), L1 repeat, *Sac*I (*S*) restriction sites, probes D1 and D2, and PCR primers (*arrowheads*) are indicated on the map. Sequence from the deletion junction revealed fusion of a poly-A/T stretch on the *COL4A6* side to the poly-A tail-derived region of the L1 repeat, with an insertion of a 70-bp stretch of poly-A/T.

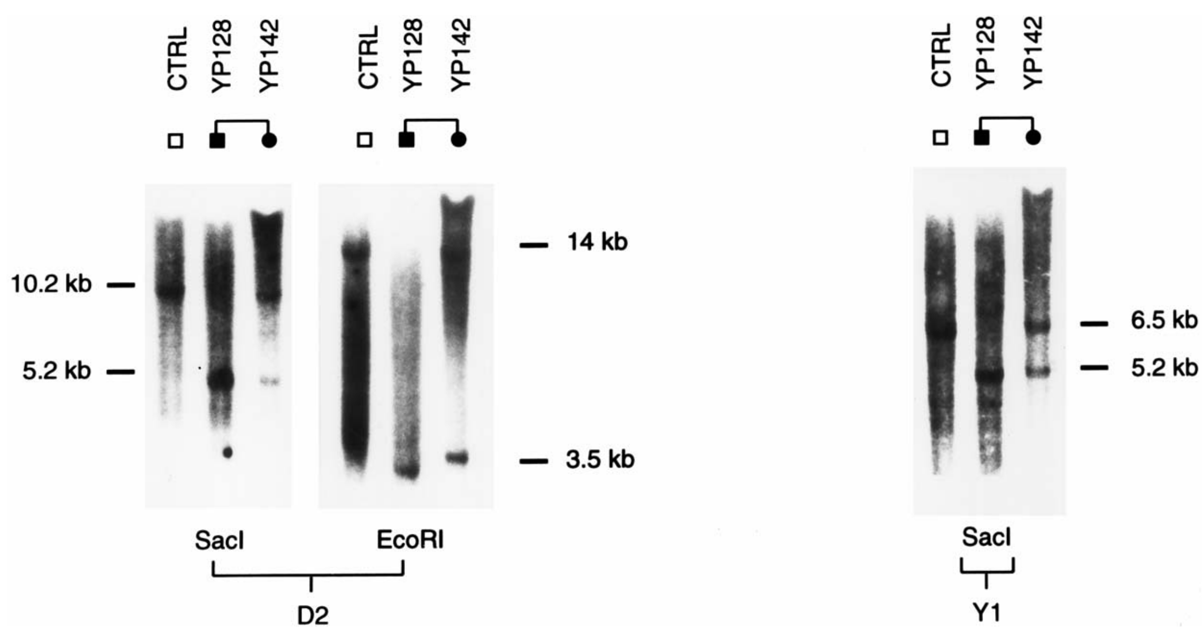

Figure 4 Southern analysis of the YP128 deletion. Probes D2 and Y1 revealed an abnormal 5.2-kb *Sac*I fragment, and probe D2 an abnormal 3.5-kb *Eco*RI fragment, in patient YP128 and his sister, YP142, compared with the control pattern (see fig. 5).

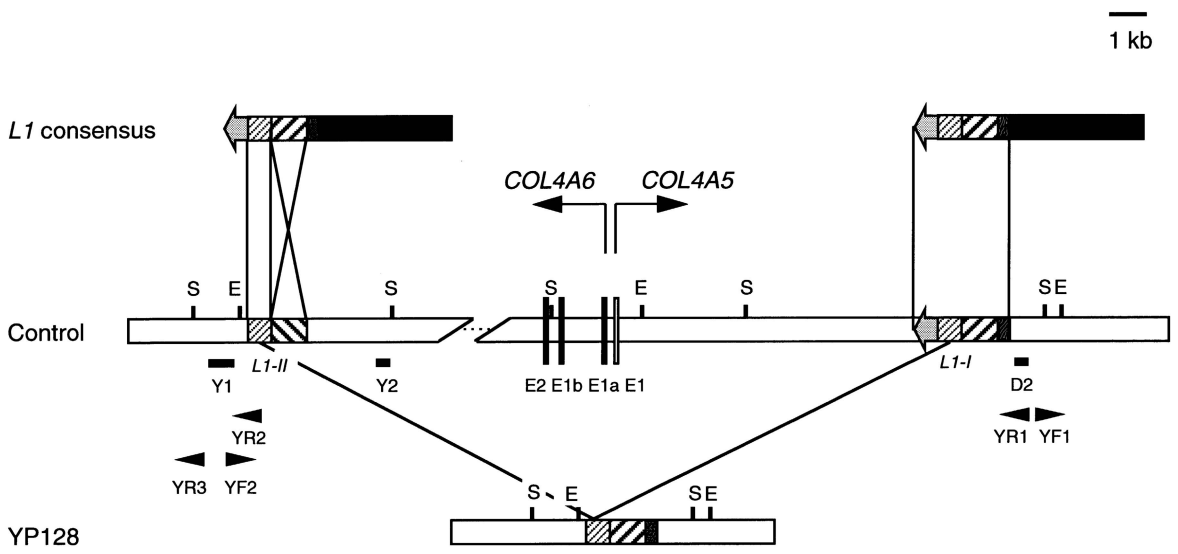

Figure 5 Deletion junction in the YP128 patient. A map containing the intergenic region (see fig. 3) and the *COL4A6* breakpoint region indicates the second L1 repeat; probes Y1, Y2, and D2; PCR primers (*arrowheads*; see Patients and Methods and table 1); and *Sac*I (*S*) and *Eco*RI (*E*) restriction sites. The L1 repeats are compared schematically against a full-length consensus element, with regions of homology filled similarly. The mutant allele arises by unequal homologous recombination between the L1 elements.

sparing of exon 3 (not shown). Using probe D2, we identified abnormal 5.2-kb *Sac*I and 3.5-kb *Eco*RI fragments (fig. 4). A probe from the *COL4A6* terminus of cosmid U230F3 (Renieri et al. 1994) did not hybridize against genomic DNA from patient YP128 (not shown), which placed the lower limit of the deletion at 40 kb. The deletion junction was isolated by PCR amplification (see Patients and Methods). Comparison with wild-type genomic DNA provided evidence for an unequal homologous recombination event involving the L1 repeat in intron 1 of *COL4A5* and a second rearranged L1 in intron 2 of *COL4A6*, the latter containing nucleotides 3621–4424 and 4405–4908 of the consensus element, in a head-to-head arrangement (fig. 5). The abnormal

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                                    COL4A6 L1   TCCTGAATGGTAGTGCCTAGGTTTTCTTCTAGGGTTTTTATGGTTTTAGGTCTA  4855
                                       YP128    TCCTGAATGGTAGTGCCTAGGTTTTCTTCTAGGGTTTTTATGGTTTTAGGTCTA
                                    COL4A5 L1   TCCTGAATGGTAATGCCTAGGTTTTCTTCTAGGGTTTTTATGGTTTTAGGTCTA
                                                                                          *
COL4A6 L1   ATGTTTCAGTCTTTAATCCATCTTGAATTAATTTTCGTATAAGGTGTAAGGAAGGGATCCAGTTTCAGCTTTCTACATATGGCTAGCCAG  4765
   YP128    ATGTTTCAGTCTTTAATCCATCTTGAATTAATTTTCGTATAAGGTGTAAGGAAGGGATCCAGTTTCAGCTTTCTACATACGGCTAGCCAG
COL4A5 L1   ACATTTAAGTCTTTAATCCATCTTGAATTAATTTTTGTATAAGGTGTAAGGAAGGGATCCAGTTTCAGCTTTCTACATATAGCGAGCCAG

COL4A6 L1   TTTTCCCAGCACCATTTATTAAATAGGGAATCCTTTCCCCATTGCTTGTTTTTCTCAGGTTTGTCAAAGATCAGATAGTTGTAGATATGT  4675
   YP128    TTTTCCCAGCACCATTTATTAAATAGGGAATCCTTTCCCCATTGCTTGTTTTTCTCAGGTTTGTCAAAGATCAGATAGTTGTAGATATGT
COL4A5 L1   TTTTCCCAGCACCATTTATTAAATAGGGAATCCTTTCCCCATTGCTTGTTTTTCTCAGGTTTGTCAAAGATCAGATAGTTGTAGATATGT

COL4A6 L1   GGCGTTATTTCTGAGGGCTCCGTTCTGTTCCATTGATCTATATCTCTGTTTTGGTACCAGTACCATGCTGTTTTGGTGACTGTAGCCTTG  4585
   YP128    GGCGTTATTTCTGAGGGCTCCGTTCTGTTCCATTGATCTATATCTCTGTTTTGGTACCAGTACCATGCTGTTTTGGTTACTGTAGCCTTG
COL4A5 L1   GGCGTTATTTCTGAGGGCTCTGTTGTTCCATTGATCTATATCTCTGTTTTGGTACCAGTACCATGCTGTTTTGGTTACTGTAGCCTTG

COL4A6 L1   TAGTATAGTTTGAAGTCAGGTAGCGTGATGCCTCCAGCTTTGTTCTTTTGGCTTAGGATTGACTTGGTGATGCGGGCTCTTTTTTGGTTC  4495
   YP128    TAGTATAGTTTGAAGTCAGGTAGCGTGATGCCTCCAGCTTTGTTCTTTTGGCTTAGGATTGACTTGGCGATGCTGGCTCTTTTTTGGTTC
COL4A5 L1   TAGTATAGTTTGAAGTCAGGTAGCATGATGCCTCCAGCTTTGTTCTTTTGGCTTAGGATTGACTTGGCGATGCTGGCTCTTTTTTGGTTC

COL4A6 L1   CATATGAACTTTAAAGTAGTTTTTTCCAATTCTGTGAAGAAAGTCATTGGTAGCTTGATGGAGATGGCATTGAATCTATAAATTGCCTTG  4405
   YP128    CATATGAACTTTAAAGTAGTTTTTTCCAATTCTGTGAAGAAAGTCATTGGTAGCTTGATGGGGACGGCATTGAATCTGTAAATTACCTTG
COL4A5 L1   CATATGAACTTTAAAGTAGTTTTTTCCAATTCTGTGAAGAAAGTCATTGGTAGCTTGATGGGGACGGCATTGAATCTGTAAATTACCTTG
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Figure 6 Recombination interval in the YP128 patient. Comparison among the wild-type *COL4A5* and *COL4A6* L1-containing breakpoint regions and the YP128 deletion junction is shown. Mismatches are shaded. With a single exception, suggesting a patient polymorphism (indicated by an asterisk [*]), mismatches segregate around the underlined interval 4518–4559. Numbering is in reverse order, corresponding to an arbitrary L1 consensus sequence (see text).

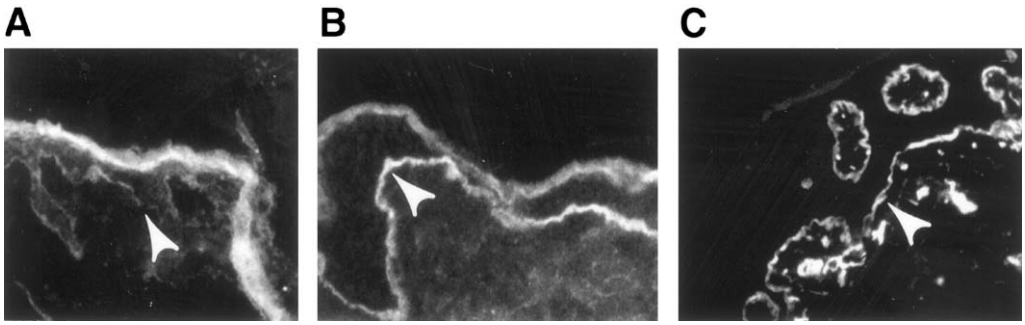

Figure 7 Immunostaining of type IV collagen chains. Epidermal basement membrane (*arrowhead*) in a skin biopsy specimen from patient YP128 did not stain with specific antibodies against the $\alpha 6$(IV) collagen chain (*a*), in contrast to a normal control (*b*). The same specimen stained positively for the $\alpha 1$(IV) collagen chain in epidermal and other skin basement membranes (*c*). Original magnification × 75.

5.2-kb *Sac*I fragment was verified by use of probe Y1 from the *COL4A6* breakpoint region (fig. 5). Using sequence comparison, we localized the recombination interval to nucleotides 4518–4559 (fig. 6). Southern analysis by use of probe Y2 from the deleted region provided no evidence of a reciprocal duplication in genomic DNA from maternally derived, transformed lymphoblasts (not shown), such as might be expected from a recombination event involving two X chromosomes in a female with somatic mosaicism.

Immunophenotypic Characterization of Patient YP128

Immunostaining of a skin biopsy specimen from patient YP128 was performed to investigate the possibility that the $\alpha 5$ and $\alpha 6$ chains of type IV collagen are expressed as disease-associated N-terminally truncated forms. Against this hypothesis, we observed the absence of the normal pattern of distribution of the $\alpha 6$ chain in the epidermal basement membrane, as compared with a normal skin sample (fig. 7; see Peissel et al. 1995). Immunoreactivity to the $\alpha 5$ chain was also lost (not shown), whereas that to $\alpha 1$ was preserved.

Discussion

Insights into the pathogenesis of smooth-muscle tumors in AS-DL are likely to have broader implications for the control of cell growth. As a first step toward uncovering its basis, we undertook detailed molecular analysis of deletions associated with AS-DL and initiated investigation of expressed sequences likely to be affected by these mutations. Our major finding is that deletions in this region arise by diverse mechanisms from resident L1 repeats. This class of repetitive elements, which has evolved from an archetypal transposable element, represents an estimated 10%–15% of the human genome (Kazazian and Moran 1998) and has been implicated in several deletion syndromes (Henthorn et al. 1990; Drechsler and Royer-Pokora 1996). It remains to be seen whether the involvement of L1 repeats in AS-DL will prove disproportionate to their representation in the locus or will extend beyond the selection bias inherent in this initial characterization of deletions.

The DON deletion, with its interposition of a 70-bp stretch of poly-A/T, is unique in its kind. Although the mechanism of the deletion is unclear, inspection of the deletion junction provides some insights. The insertion is flanked on both sides by A/T-rich regions (fig. 3). If these arose as overhangs after double-stranded breaks, then repair might have been accomplished by oligonucleotide capture, wherein a preformed oligonucleotide—likely a poly-A tail—served in a bridging intermediate structure, with the subsequent generation of a junction by means of filling in and nick ligation. In principle, a repair mechanism using "filler DNA" (Roth et al. 1989) could have occurred in the context of an intra- or interchromosomal event in the maternal germ line. Precedent for an intrachromosomal event is provided by an earlier description of a male with AS-DL, who showed somatic mosaicism for a pathogenic deletion, also involving the L1 repeat in intron 1 of *COL4A5* (Ueki et al. 1998). Interestingly, breakpoints in this deletion arose at topoisomerase I and II consensus-binding sites, which led to their direct implication in the event. In the DON deletion junction no such sites are evident, although clearly this does not exclude the involvement of these enzymes.

To our knowledge, the deletion in the YP128 family represents only the second reported deletion arising from an unequal homologous recombination between neighboring L1 elements. In the first, the recombination event gave rise to a 7.5-kb deletion in the phosphorylase kinase β–subunit gene, which included a single coding exon, and caused glycogen storage disease in a homozygous male born to consanguineous parents (Burwinkel and Kilimann 1998). That these events are detected rarely,

in spite of the abundance of L1 elements in the human genome, has been pointed out by others, who have also posed possible explanations, among them: (1) that these repeats may occur preferentially in gene-poor regions, such that recombination events are clinically silent; (2) that frequent and extensive mutations over evolution have limited the homology among elements; and (3) that, in spite of their abundance, L1 elements occur at infrequent intervals, rendering recombinations involving colinear elements unlikely (Burwinkel and Kilimann 1998). It will be interesting to ascertain whether exceptions to these general postulates apply in the *COL4A5-COL4A6* locus, favoring this as a hot spot for deletions. Further, it is tempting to speculate that these or other as yet uncharacterized repetitive elements may have played a role in the original duplications giving rise to the type IV collagen gene family (Zhou et al. 1994). Insight into this possibility will require broader knowledge of the *COL4A1-COL4A2* and *COL4A3-COL4A4* loci.

The characterization of these deletions does not allow us to distinguish among the possible mechanisms of tumorigenesis in AS-DL (see Introduction). In the YP128 patient, we found loss of $\alpha 5$ and $\alpha 6$ immunoreactivity, which is consistent with findings in other affected individuals (Heidet et al. 1997*a,* 1998) and provides evidence against the role(s) of N-terminally truncated type IV collagen chains in this syndrome. Loss of the $\alpha 5$ and $\alpha 6$ chains per se is not likely sufficient to cause the disease, as this is also the prevalent finding in AS attributable to mutations in the *COL4A5* gene alone (Peissel et al. 1995). Of course, loss of type IV collagen chains could be a contributing factor, as there is evidence of a disordered substratum in esophageal leiomyomas by morphological and immunocytochemical criteria (Heidet et al. 1997*a*). It is possible that the subunit composition of type IV collagen in affected individuals favors incremental derangements in AS-DL as compared to AS (e.g., if homotrimers of the $\alpha 6$ chain occur and are preserved selectively in AS). We remain intrigued by the possibility that additional genes are contained within the *COL4A5-COL4A6* locus, which confers gain of function, causing smooth-muscle tumors, and that these additional genes are rendered functionally null by deletions extending beyond exon 3 of *COL4A6*. It is noteworthy that these genes would be subject to disruption in deletions as small as 13.4 kb.

To summarize, we have characterized the molecular features of two pathogenic deletions in AS-DL. Comparison of these deletions provides evidence of their having arisen by two different mechanisms, involving nonhomologous recombination in the DON patient and an unequal homologous recombination in the YP128 family. The participation of L1 repeats emphasizes their importance—perhaps as vestiges of a primordial duplication event—and raises the possibility that the emergence of this locus as a hot spot for recombination may have followed from their strategic localization.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for full-length L1 element containing two open reading frames [U93574])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/htbin-post/Omim (for Alport syndrome [MIM 104200] and Alport syndrome, X-linked [MIM 301050])

References

Alport AC (1927) Hereditary familial congenital haemorrhagic nephritis. Br Med J 1:504–506

Ausubel FM, Brent R, Kingston, RE, Moore, DD, Seidman JG, Smith JA, Struhl, K (eds) (1994) Current protocols in molecular biology. Greene Publishing and Wiley-Interscience, New York

Burwinkel B, Kilimann MW (1998) Unequal homologous recombination between LINE-1 elements as a mutational mechanism in human genetic disease. J Mol Biol 277: 513–517

Cochat P, Guibaud P, Garcia Torres R, Roussel B, Guarner V, Larbre F (1988) Diffuse leiomyomatosis in Alport syndrome. J Pediatr 113:339–343

Dracopoli NC, Haines JL, Korf BR, Moir DT, Morton CC, Seidman CE, Seidman JG, et al (eds) (1997) Current protocols in human genetics. John Wiley & Sons, New York

Drechsler M, Royer-Pokora B (1996) A LINE element is present at the site of a 300-kb deletion starting in intron 10 of the PAX6 gene in a case of familial aniridia. Hum Genet 98:297–303

Garcia-Torres R, Orozco L (1993) Alport-leiomyomatosis syndrome: an update. Am J Kidney Dis 22:641–648

Gregory MC, Atkin CL (1993) Alport Syndrome. In: Schrier RW, Gottschalk CW (eds) Disease of the kidney. Vol. 1. Little, Brown, Boston, pp 571–591

Hasstedt SJ, Atkin CL, San Juan AC, Jr. (1986) Genetic heterogeneity among kindreds with Alport syndrome. Am J Hum Genet 38:940–953

Heidet L, Boye E, Cai Y, Sado Y, Zhang X, Flejou JF, Fekete F, et al (1998) Somatic deletion of the 5′ ends of both the COL4A5 and COL4A6 genes in a sporadic leiomyoma of the esophagus. Am J Pathol 152:673–678

Heidet L, Cai Y, Sado Y, Ninomiya Y, Thorner P, Guicharnaud L, Boye E, et al (1997*a*) Diffuse leiomyomatosis associated with X-linked Alport syndrome: extracellular matrix study

using immunohistochemistry and in situ hybridization. Lab Invest 76:233–243

Heidet L, Cohen-Solal L, Boye E, Thorner P, Kemper MJ, David A, Larget Piet L, et al (1997*b*) Novel COL4A5/COL4A6 deletions and further characterization of the diffuse leiomyomatosis-Alport syndrome (DL-AS) locus define the DL critical region. Cytogenet Cell Genet 78:240–246

Heidet L, Dahan K, Zhou J, Xu Z, Cochat P, Gould JDM, Leppig KA, et al (1995) Deletions of both $\alpha 5$(IV) and $\alpha 6$(IV) collagen genes in Alport syndrome and in Alport syndrome associated with smooth muscle tumours. Hum Molec Genet 4:99–108

Henthorn PS, Smithies O, Mager DL (1990) Molecular analysis of deletions in the human β-globin gene cluster: deletion junctions and locations of breakpoints. Genomics 6: 226–237

Hudson BG, Reeders ST, Tryggvason K (1993) Type IV collagen: structure, gene organization, and role in human diseases: molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis. J Biol Chem 268: 26033–26036

Kazazian HH, Jr., Moran JV (1998) The impact of L1 retrotransposons on the human genome. Nat Genet 19:19–24

Kleppel MM, Kashtan C, Santi PA, Wieslander J, Michael AF (1989) Distribution of familial nephritis antigen in normal tissue and renal basement membranes of patients with homozygous and heterozygous Alport familial nephritis: relationship of familial nephritis and Goodpasture antigens to novel collagen chains and type IV collagen. Lab Invest 61: 278–289

Nakanishi K, Yoshikawa N, Iijima K, Kitagawa K, Nakamura H, Ito H, Yoshioka K, et al (1994) Immunohistochemical study of $\alpha 1$-5 chains of type IV collagen in hereditary nephritis. Kidney Int 46:1413–1421

Peissel B, Geng L, Kalluri R, Kashtan C, Rennke HG, Gallo GR, Yoshioka K, et al (1995) Comparative distribution of the $\alpha 1$(IV), $\alpha 5$(IV), and $\alpha 6$(IV) collagen chains in normal human adult and fetal tissues and in kidneys from X-linked Alport syndrome patients. J Clin Invest 96:1948–1957

Renieri A, Bassi MT, Galli L, Zhou J, Giani M, De Marchi M, Ballabio A (1994) Deletion spanning the 5′ ends of both the COL4A5 and COL4A6 genes in a patient with Alport's syndrome and leiomyomatosis. Hum Mutat 4:195–198

Roth DB, Chang XB, Wilson JH (1989) Comparison of filler DNA at immune, nonimmune, and oncogenic rearrangements suggests multiple mechanisms of formation. Mol Cell Biol 9:3049–3057

Sassaman DM, Dombroski BA, Moran JV, Kimberland ML, Naas TP, DeBerardinis RJ, Gabriel A, et al (1997) Many human L1 elements are capable of retrotransposition. Nat Genet 16:37–43

Timpl R, Brown JC (1996) Supramolecular assembly of basement membranes. Bioessays 18:123–132

Triglia T, Peterson MG, Kemp DJ (1988) A procedure for in vitro amplification of DNA segments that lie outside the boundaries of known sequences. Nucleic Acids Res 16:8186

Tryggvason K (1996) Mutations in type IV collagen genes and Alport phenotypes. Contrib Nephrol 117:154–171

Ueki Y, Naito I, Oohashi T, Sugimoto M, Seki T, Yoshioka H, Sado Y, et al (1998) Topoisomerase I and II consensus sequences in a 17-kb deletion junction of the *COL4A5* and *COL4A6* genes and immunohistochemical analysis of esophageal leiomyomatosis associated with Alport syndrome. Am J Hum Genet 62:253–261

Zhou J, Ding M, Zhao Z, Reeders ST (1994) Complete primary structure of the sixth chain of human basement membrane collagen, $\alpha 6$(IV): isolation of the cDNAs for $\alpha 6$(IV) and comparison with five other type IV collagen chains. J Biol Chem 269:13193–13199

Zhou J, Mochizuki T, Smeets H, Antignac C, Laurila P, de Paepe A, Tryggvason K, et al (1993) Deletion of the paired $\alpha 5$(IV) and $\alpha 6$(IV) collagen genes in inherited smooth muscle tumors. Science 261:1167–1169